Use of the Relative Dose Response (RDR) Assay to Determine Vitamin A Status of Calves at Birth and Four Weeks of Age[1,2]

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ABSTRACT

An accurate assessment of vitamin A status can be determined by analysis of liver biopsy samples; however, liver biopsies are not always feasible. Plasma concentrations of vitamin A do not provide an accurate indication of vitamin A status. The objective of this study, therefore, was to determine the ability of the relative dose response assay to indicate the vitamin A status of Holstein calves. Calves were obtained at birth and assigned to vitamin A treatments (0, 1700, 34,000, or 68,000 IU/d) added to milk replacer. Liver biopsies and relative dose response assays were performed at birth and 4 wk. Calves supplemented with 1700, 34,000, or 68,000 IU of vitamin A/d had adequate (greater than 20 μg/g) liver concentrations of vitamin A at 4 wk of age. The relative dose response assay at 4 wk was correlated with liver concentrations of vitamin A. Both the relative dose response assay and liver concentrations of vitamin A indicated that calves not supplemented with vitamin A had low vitamin A status, whereas other treatment groups had adequate vitamin A status. Plasma concentrations of retinol increased by 4 wk of age in calves receiving supplemental vitamin A at 34,000 IU and 68,000 IU/d and decreased in unsupplemented calves; however, all calves had concentrations of <20 μg of retinol/dl of plasma. The relative dose response assay agreed with liver biopsies as an indication of vitamin A status, whereas plasma concentrations of retinol incorrectly indicated all treatment groups were deficient in vitamin A.
(**Key words:** calves, vitamin A, relative dose response assay)

Abbreviation key: RDR = relative dose response

INTRODUCTION

Liver concentrations of vitamin A are the most reliable indication of the true vitamin A status of an individual with 20 μg of vitamin A/g of liver considered a concentration that is adequate (25). A concentration of less than 20 μg of retinol/dl of plasma has been used as an indication of vitamin A deficiency in cattle (12); however, this criterion for deficiency may not apply to young calves (10). Additionally, the concentration of retinol in plasma is not a reliable indication of vitamin A status. The liver regulates circulating retinol and maintains concentrations within a narrow range until the liver is nearly depleted (26). Because liver biopsies are an invasive procedure and plasma concentrations are unreliable, the relative dose response **(RDR)** assay has been used with several species to determine vitamin A status (3, 11, 16). When liver vitamin A is deficient, retinol binding protein (the transport protein for retinol) accumulates in the liver (17). After administration of a dose of vitamin A, concentrations of retinol in plasma increase because retinol binding protein is readily available to transport retinol from the liver into the bloodstream. A common method for determining the RDR in humans is to obtain a baseline blood sample, administer an oral dose of vitamin A (normally 450 retinol equivalents for adults), then obtain a second blood sample 5 h postdosing, when concentrations of retinol peak in plasma. Amedee-Manesme et al. (3) consider an increase in plasma concentrations of retinol greater than 20% above baseline values to indicate that liver concentrations of vitamin A are deficient

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(less than 20 μg/g). Alternatively, an increase of less than 10% indicates liver concentrations of vitamin A likely are adequate. The authors did not make a conclusion regarding RDR values between 10 and 20%.

The RDR assay has been used as an indication of the vitamin A status of humans (3), rats (16), horses (14), and sheep (8). A modified RDR assay was used with cattle greater than 6 mo of age as a tool for studying vitamin A metabolism (27). For cattle and sheep, the postdosing samples were obtained at 20 h (27) and 12 h (8), respectively, to allow for passage of vitamin A through the rumen. Neonatal calves were investigated as a model for development of the RDR assay as an indication of vitamin A status of cattle because calves are born with low concentrations of vitamin A (<8 μg/g) in liver (7). The RDR assay did not correctly identify low concentrations of vitamin A in liver of neonatal calves, however. Changes in plasma retinol of neonatal calves were related to the concentration of vitamin A in colostrum rather than the concentration of vitamin A in liver. Research (10) indicates that calves at 4 wk of age have retinol concentrations in plasma (approximately 15 μg/dl) that are still below concentrations normally considered adequate even with vitamin A supplementation as high as 30,000 IU/d. The objective of this study, therefore, was to evaluate the feasibility of using the RDR assay to determine the vitamin A status of calves fed 0, 1700, 34,000, or 68,000 IU of vitamin A/d through 4 wk of age.

MATERIALS AND METHODS

Calf Procedures

Male Holstein calves (n = 53) were used to study the ability of the RDR assay to determine the vitamin A status of young calves. Calves were purchased from two commercial dairies. Twenty-four calves completed the study between August 28, 1996, and December 5, 1996, and were designated fall calves. The remainder of the calves completed the study between April 26, 1997, and July 30, 1997, and were designated spring calves. Calves were isolated from the dam immediately postpartum and brought to the South Dakota State University Dairy Research and Teaching Facility, where they were housed outdoors in individual calf hutches. Calves were randomly assigned to treatments of 0, 1700 (approximate NRC recommendation [18]), 34,000 (approximate supplementation in commercial milk replacers), or 68,000 (potentially toxic level of supplementation [18]) IU of vitamin A/d. Vitamin A was in the form of a water dispersible retinyl acetate (Microvit A Prosol 500; Rhone Poulenc, Atlanta, GA). All animal procedures were approved by the Institutional Animal Care and Use Committee.

Colostrum was collected, pooled, and frozen before each portion of the trial. Colostrum was thawed and heated in a warm water bath before feeding to calves. If a calf did not suckle, colostrum was fed via an esophageal feeder. Samples of colostrum were obtained randomly throughout the trial to insure vitamin A concentrations in colostrum were consistent.

Upon arrival at the South Dakota State University dairy farm, and prior to feeding colostrum, calves were weighed and a modification of the RDR assay was performed. Blood samples were obtained by jugular venipuncture into Vacutainers (Becton Dickinson Vacutainer Systems, Rutherford, NJ) containing 143 units of sodium heparin. Samples were placed on ice and kept in the dark until centrifugation for recovery of plasma. Liver biopsies were performed and calves subsequently were fed pooled colostrum, at 5% of BW, within 3.2 ± 1.5 h (range, 1.5 to 9.5 h) after birth. Vitamin A supplied naturally by the colostrum exceeded 450 retinol equivalents and was considered the dose of vitamin A for the modified RDR assay. Blood samples were obtained again at 20 h after feeding colostrum based on studies conducted by Boner (5) and Westendorf et al. (27). Boner (5) reported that during the first 24 h after consuming colostrum, plasma retinol concentrations in calves peaked at 20 h. Westendorf et al. (27) reported that plasma concentrations of retinol peaked at 20 h after feedlot cattle consumed a large dose of vitamin A.

Calves were fed, at 5% of BW, reconstituted milk replacer (Milk Specialities Co., Dundee, IL) supplemented with half the daily treatment of vitamin A beginning at 12 h after the first feeding. Milk replacer was an all milk-protein product formulated to contain 20% protein and 20% fat, without supplemental vitamins A or E. After the first 20 h, calves were fed milk replacer with half the daily supplementation of vitamin A at approximately 0630 and 1830 h daily.

Calves were weighed on d 7, 14, 21, and 28 after birth. Each week the amount of milk replacer fed daily was adjusted to 10% of BW. Fresh water was offered daily after the morning feeding beginning on d 4. Calf starter was not offered during the trial.

The RDR assay was performed again at 4 wk of age. Initial blood samples and liver biopsy samples were obtained after calves were fasted for at least 8 h. Calves subsequently were fed milk replacer containing a dose of 1700 IU (585 μg) of vitamin A as retinyl acetate. Postdosing samples were obtained at 4, 6, and 8 h to encompass sample times used with humans (3), rats (16), and horses (14). Subsequently, calves were fed milk replacer without vitamin A at the evening

feeding. Postdosing samples also were obtained at 20 h because of results obtained by others (5, 27).

Liver biopsies at birth and 4 wk were performed according to the procedure of Boner (5). Briefly, an area 15 cm below the topline, between the 10th and 13th ribs, was shaved and cleaned with ethanol. Lidocaine (1 cc) was injected intramuscularly at the site of the incision between the 11th and 12th ribs. A 2.5-cm incision was made and a 14-mm Bauer one-handed biopsy needle (Products Group International, Inc., Boulder, CO) was inserted at approximately a 45° angle toward the point of the left shoulder. Two samples, (averaging 15 mg/sample) were obtained, placed on ice, and kept in the dark. A 7% iodine solution was poured over the incision area. Liver samples were stored at –20°C until analysis for vitamin A (9).

Health of calves was monitored daily. Fecal scores were recorded twice daily based on methods described by Larson et al. (15). Briefly, feces were scored from 1 through 4 for fluidity with 1 as normal and 4 as watery. Rectal temperatures were obtained for calves having a fecal score ≥ 3. Calves with a fecal score of ≥ 3 for two consecutive days were treated with electrolytes (Biolyte; Pharmacia & UpJohn, Kansas City, MO) after each milk replacer feeding until fecal scores were 2 or lower. Rectal temperatures were monitored at each feeding until the temperature of the calf returned to normal. Calves were observed weekly for signs of vitamin A deficiency. Signs considered indicative of possible vitamin A deficiency were rough hair coat, incoordination, watery eyes, scaly skin, cloudy corneas, and blindness.

Laboratory Procedures

Vitamin A (alcohol and esters) in colostrum samples was analyzed by spectrophotometry with a modification of a previous method (9). Colostrum samples were placed in a water bath under yellow light at 37°C until the milk fat was heated. Colostrum was mixed thoroughly and 1 ml was added to ethanol (1 ml) and petroleum ether (5 ml). The mixture was vortexed and centrifuged (250 × *g*, 21°C, 2 min). The top layer was removed and dried, in the dark, with a heated (43°C) centrifuge (Savant Speed Vac Plus SC 110A, Farmingdale, NY). The residue was reconstituted with chloroform (1.5 ml). Trifluoroacetic acid (1.5 ml) was added and the absorbance of the mixture, at 616 nm visible light, was determined within 10 s (Beckman DU-50 Spectrophotometer, Fullerton, CA). Milk replacer samples were analyzed for vitamin A and β-carotene by HPLC (20).

Vitamin A (alcohol and esters) in liver samples was extracted and prepared for spectrophometric analysis by a modification of a previous procedure (1). Briefly, liver samples were weighed and combined with anhydrous sodium sulfate (1.5 g) and methylene chloride (12 ml). The mixture was ground with a mortar and pestle under yellow light and subsequently filtered through a 60-ml Pyrex funnel with a fritted disc (Fisher; Itasca, IL). The amount of methylene chloride recovered was recorded. Butylated hydroxytoluene (0.5 mg) and ethanol (0.1 ml) were added. The mixture was dried in a heated centrifuge and vitamin A content was determined as described for colostrum.

Blood samples were centrifuged (1000 x *g*, 4°C, 20 min) and plasma, harvested under yellow light, was stored at –20°C until vitamin analysis. The concentration of retinol in plasma samples was determined by HPLC (SP 8800 ternary HPLC pump, Spectra 100 variable UV-Vis wavelength detector, and Chrom Jet Integrator SP 4400, Spectra Physics, San Jose, CA) using the procedure of Bieri et al. (4). Three standards—high, medium, and low concentrations (Sigma, St. Louis, MO)—were run for 10 min. The low standard contained (per ml) 0.36 μg of retinol, 1.16 μg of retinyl acetate, and 2.546 μg of RRR α-tocopherol. The medium standard contained (per ml) 0.72 μg of retinol, 1.16 μg of retinyl acetate, and 5.09 μg of RRR α-tocopherol. The high standard contained (per ml) 1.08 μg of retinol, 1.16 μg of retinyl acetate, and 10.18 μg of RRR α-tocopherol.

Statistical Analysis

Data for liver and plasma vitamin A concentrations were analyzed by the mixed model procedure of SAS (22) using repeated measures analysis. Data for birth and 4 wk are presented as actual means ± SEM. Season, treatment, and sample were tested as main effects. Batch of milk replacer within season was included in the model because the last 10 calves in the spring were fed a different batch. Interactions among season, treatment, and sample were tested in the model.

Correlations between the concentration of vitamin A in the liver and the RDR assay at 4, 6, 8, and 20 h postdosing with vitamin A at 4 wk of age were investigated using the PROC CORR procedure of SAS.

RESULTS

Samples of pooled colostrum contained fairly consistent concentrations of vitamin A (alcohol and esters), ranging from 211.3 to 258.4 μg/dl. Concentrations of vitamin A in colostrum were similar to mean concentrations of 218.4 ± 109 μg/dl (5) and 440 ± 334 μg/dl (6) reported by others. Two batches of milk replacer

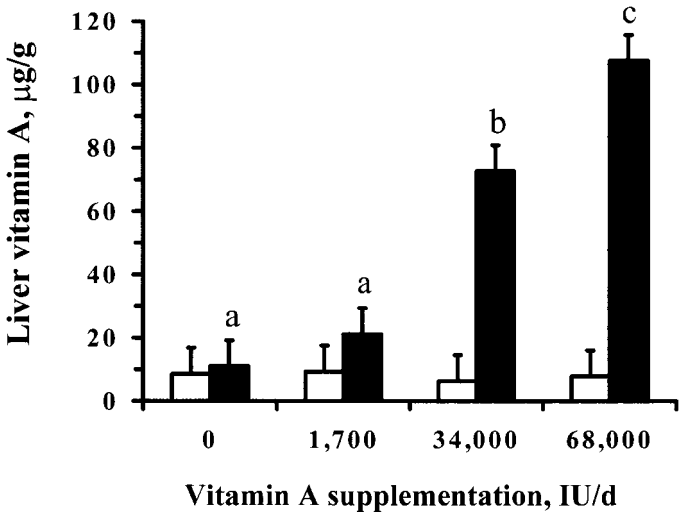

Figure 1. Concentrations of vitamin A in liver samples at birth (open bars) and 4 wk (solid bars) from calves supplemented with 0, 1700, 34,000, or 68,000 IU of vitamin A daily. Liver samples were obtained prior to administration of a challenge dose of vitamin A for the relative dose response (RDR) assay. Data are presented as means ± SEM. For 4-wk samples, bars with different letters differ ($P < 0.05$).

were fed during the trial. Batch 1 contained the equivalent of 506 IU of endogenous vitamin A (vitamin A and β- carotene)/kg of milk replacer, whereas batch 2 contained the equivalent of 2372 IU of endogenous vitamin A/kg of milk replacer. Batch 1 provided approximately 230 IU of vitamin A/d and batch 2 provided approximately 1000 IU of vitamin A/d to calves in addition to the assigned treatment. Because of differences in endogenous vitamin A content of the milk replacer, batch of milk replacer was included in the statistical model.

The mean concentration of vitamin A (alcohol plus esters) in liver samples at birth was 7.9 μg/g (wet basis) and concentrations were not different among treatment groups (Figure 1). Treatment means of concentrations of vitamin A in livers at birth ranged from 6.3 ± 4.2 to 9.3 ± 8.5 μg/g of liver. By 4 wk of age, concentrations of vitamin A in liver samples from calves reflected the respective amounts of supplemental vitamin A (Figure 1). The concentration of vitamin A in liver samples increased ($P = 0.0002$) as supplementation increased. Additionally, there was an effect of batch of milk replacer as spring calves fed milk replacer from batch 2 had greater ($P < 0.01$) concentrations of vitamin A in liver samples than spring calves fed milk replacer from batch 1 or fall calves. Mean concentrations of vitamin A in liver samples at 4 wk were 11.0, 21.1, 72.6, and 107.4 μg/g for calves supplemented with vitamin A at 0, 1700, 34,000, and 68,000 IU/d, respectively. At 4 wk of age, mean concentrations of vitamin A in liver samples from calves supplemented with vitamin A at 34,000 and 68,000 IU/d were greater ($P = 0.0001$) than mean concentrations of vitamin A in liver samples at birth. Calves supplemented with vitamin A at 34,000 or 68,000 IU/d also had greater mean concentrations of vitamin A in liver samples at 4 wk of age than did calves supplemented with vitamin A at 0 or 1700 IU/d.

The mean RDR values at 20 h after colostrum feeding (8 h after initiation of vitamin A supplementation) increased ($P < 0.05$) with increasing levels of vitamin A supplementation. Values were 6.8, 23.0, 44.3, and 72.6% for the 0, 1700, 34,000, and 68,000 IU/d treatments, respectively.

At 4 wk of age, the group of calves receiving no supplemental vitamin A had mean RDR values at 6 h postdosing that were greater than 20% and 8 h postdosing values greater than 15%. All RDR values for the groups of calves fed supplemental vitamin A (1700, 34,000, or 68,000 IU/d) were less than 10% (Figure 2). For the group of calves not fed supplemental vitamin A, mean plasma concentrations of retinol peaked at 6 h (Figure 2B), and were still high at 8 h (Figure 2C), postdosing with vitamin A. The 4-(Figure 2A) and 20-h (Figure 2D) RDR values were positive for the group of calves not supplemented with vitamin A but were less than 15%, whereas the RDR values for the groups of calves supplemented with vitamin A were negative. For the group of calves not fed supplemental vitamin A, the 4-wk RDR values obtained at 6 and 8 h postdosing were negatively correlated ($r = -0.42$, $P < 0.003$) with liver concentrations of vitamin A at 4 wk.

Mean concentrations of retinol in plasma of calves at birth and 4 wk are presented in Figure 3. Mean concentrations of vitamin A in plasma at birth were similar ($P > 0.05$) among treatment groups. Mean concentrations ranged from 6.1 ± 2.8 to 7.3 ± 4.9 μg/dl (Figure 3). By 4 wk of age, the mean concentration of vitamin A in plasma of calves not supplemented with vitamin A had decreased ($P < 0.05$) from 7.3 μg/dl to 4.6 μg/dl. The mean concentrations of retinol in plasma of other treatment groups did not differ ($P > 0.05$; 1700 IU/d) from birth values, or increased ($P = 0.0001$; 34,000 or 68,000 IU/d) compared to birth values (Figure 3). The group of calves supplemented with 0 IU of vitamin A/d tended ($P = 0.058$) to have lower mean concentrations of retinol in plasma compared with the group of calves supplemented with 1700 IU of vitamin A/d. At 4 wk of age, groups of calves supplemented with 0 or 1700 IU/d had lower ($P < 0.01$) mean concentrations of retinol in plasma than calves supplemented with 34,000 and 68,000 IU/d. There was no effect ($P > 0.05$) of season or batch of milk replacer on mean concentrations of retinol in plasma.

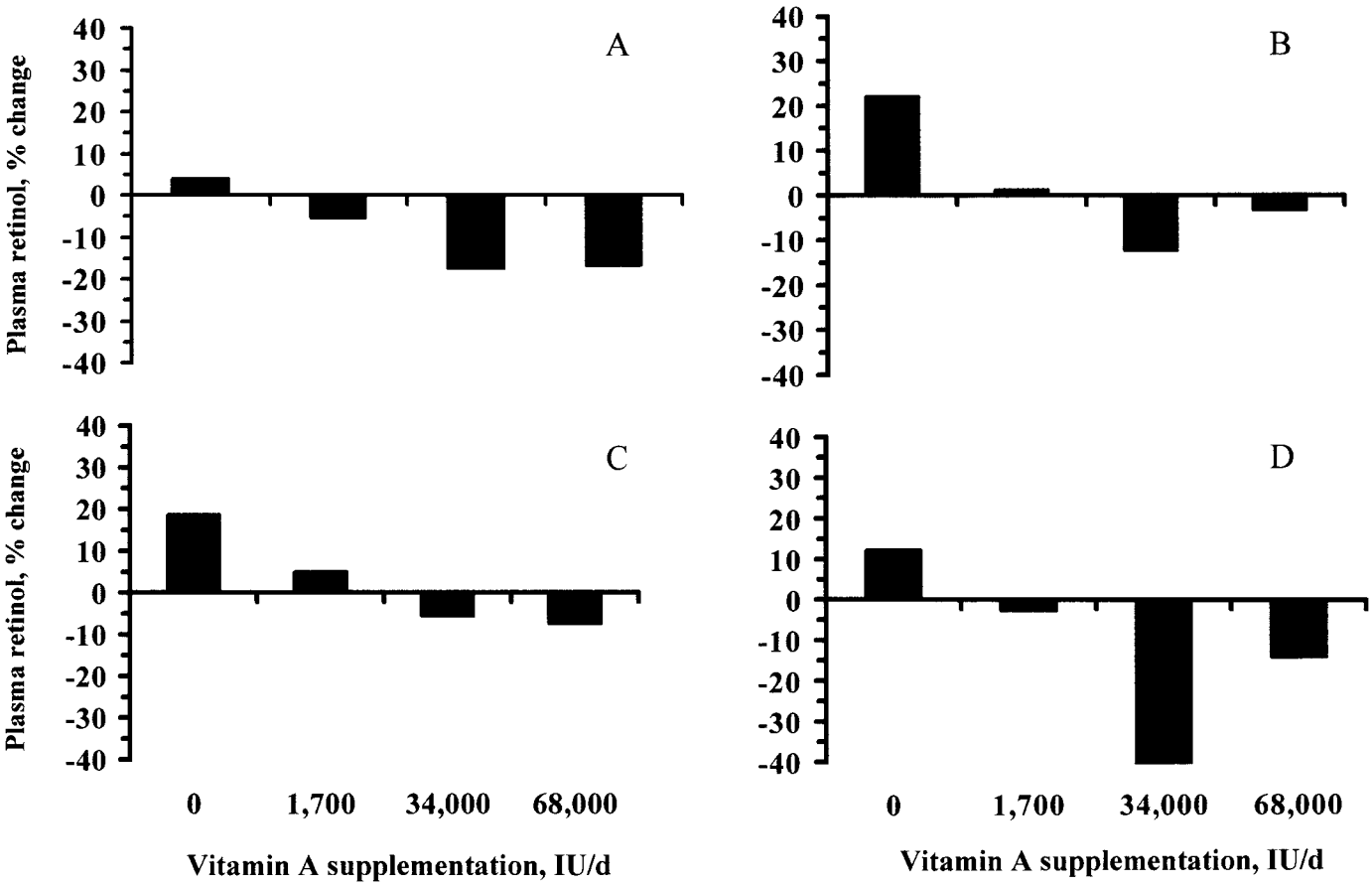

Figure 2. Relative dose response (RDR) assay for 4-wk-old calves at 4 (A), 6 (B), 8 (C), and 20 (D) h after dosing with 1700 IU of vitamin A as retinyl acetate. Calves were supplemented daily from birth to 4 wk with 0, 1700, 34,000, or 68,000 IU of retinyl acetate. Data are presented as percentage of change in plasma concentrations of retinol. Increases in plasma concentrations of retinol ≥ 20% indicate vitamin A deficiency.

DISCUSSION

This study is the first comparison of liver vitamin A concentrations with the RDR assay in young calves. It also provides important new information regarding effects of supplementation with vitamin A on the vitamin A status of young calves. The mean concentration of vitamin A in liver samples at birth was similar to values of 7.7 μg/g and 7.3 μg/g reported by Boner (5) and Branstetter et al. (7), respectively. The concentration of vitamin A in liver of calves at birth is naturally low because of little transfer of vitamin A across the placenta from the dam (under normal supplementation of vitamin A) to the fetus prior to birth (23). Substantially increasing the concentration of vitamin A present in liver of calves at birth has required supplementation of the dam with large amounts of vitamin A (1×10^6 IU daily) for 60 d prior to parturition (23). Low storage of vitamin A in liver of calves at birth (even below 10 μg/g) apparently is normal and may be a protective mechanism to prevent teratogenic effects of high intake of vitamin A by dams. None of the calves had signs of vitamin A deficiency, such as weakness or blindness, at birth. Thus, the criterion of less than 20 μg of vitamin A per g of liver as an indicator of vitamin A deficiency is not valid for newborn calves.

The mean concentration of vitamin A in liver samples at 4 wk of age from calves not supplemented with vitamin A was similar to the mean concentration of vitamin A in liver samples obtained at birth. The lack of a decrease in liver stores of vitamin A is likely attributable to consumption of colostrum at birth. Colostrum provided approximately 16,000 IU of vitamin A (not including β-carotene) in one dose. Parrish et al. (19) estimated that the apparent absorption by calves of vitamin A from colostrum was 81 to 95%. Additionally, vitamin A and β-carotene supplied by the milk replacer may have helped prevent a decline in liver vitamin A. Calves not supplemented with additional vitamin A had mean liver concentrations of vitamin A at 4 wk

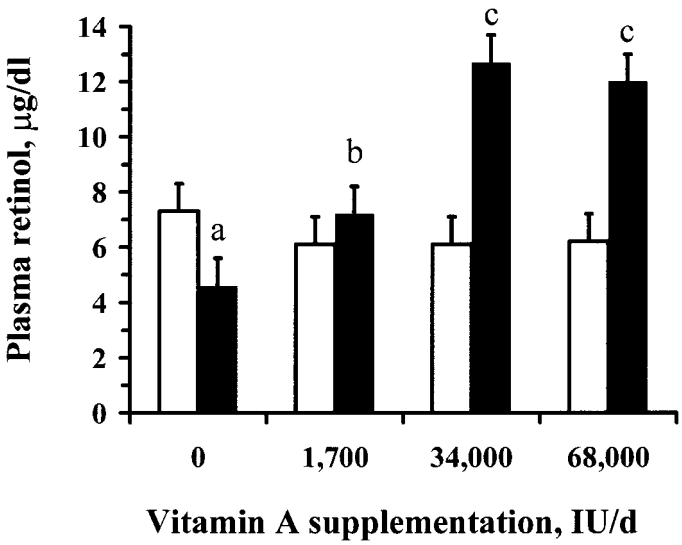

Figure 3. Concentrations of retinol in plasma samples at birth (open bars) and 4 wk (solid bars) from calves supplemented with 0, 1700, 34,000 or 68,000 IU of vitamin A daily. Data are presented as means ± SEM. For 4-wk samples, bars with different letters differ ($P < 0.05$).

of age less than 20 μg/g; however, calves did not exhibit clinical signs of vitamin A deficiency such as rough hair coat, decreased intake, incoordination, cloudy corneas or blindness.

Mean concentrations of vitamin A in liver samples of calves supplemented with vitamin A increased as the amount of supplementation increased. By 4 wk of age, mean concentrations of vitamin A were greater than 20 μg/g of liver for calves supplemented with 1700, 34,000, or 68,000 IU of vitamin A daily. No clinical signs of deficiency or toxicity were observed. One calf supplemented at 68,000 IU of vitamin A daily developed pneumonia and subsequently had low weight gains throughout the trial. It is unknown if susceptibility to pneumonia was related to excessive vitamin A intake.

Similar to results obtained by Boner (5), the modified RDR assay at birth did not provide an indication of the vitamin A status of calves. Boner (5) reported that the RDR assay at birth reflected the concentration of vitamin A in colostrum. For the present study, pooled colostrum was fed at birth so that all calves received essentially the same amount of vitamin A. Supplementation of the various levels of vitamin A began at the second feeding. The RDR at 20 h after birth, therefore, reflected the amount of vitamin A supplemented at the second feeding rather than the vitamin A status. The increases in plasma concentrations of retinol at 20 h after birth in calves supplemented with 34,000 and 68,000 IU of vitamin A/d also indicates that retinol binding protein likely is not a limiting factor in increasing circulating vitamin A. Retinol binding protein evidently is present in the liver of calves at birth, or shortly after birth, and is available to transport retinol from the liver.

The RDR assay at 4 wk of age, considering 6-h postdosing values, indicated that calves not fed supplemental vitamin A were deficient in vitamin A (RDR values ≥20%), whereas calves fed supplemental vitamin A were not deficient (RDR values < 10%). The 6-h postdosing sample provided a more accurate indication of vitamin A status than the 4-, 8-, or 20-h samples. The 4- (Figure 2A), 8- (Figure 2C) and 20-h (Figure 2D) RDR assay values were less definitive for determination of vitamin A status because, although the group of calves not supplemented with vitamin A was the only group with positive RDR values, the RDR values were less than 20%.

This is the first report of the use of the RDR assay to determine the vitamin A status of young calves. It is also the first study to correlate the RDR assay with liver concentrations of vitamin A. Westendorf et al. (27) used a modification of the RDR assay to evaluate metabolism of vitamin A in feedlot cattle. The authors dosed cattle with 20, 30, or 40 times the daily requirement of vitamin A to determine how much vitamin A was required to increase retinol in plasma and when vitamin A would peak in plasma of cattle after dosing. Westendorf et al. (27) reported that a dose 30 times the requirement for vitamin A (approximately 700,000 IU) causes increases in plasma vitamin A of feedlot cattle by 20-h postdosing. In contrast, in the current study, increases in plasma retinol occurred in young calves dosed with 1700 IU by 6-h postdosing when the calves were deficient in vitamin A. The study conducted by Westendorf et al. (27) was designed to investigate metabolism of vitamin A rather than to determine the ability of the RDR assay to indicate vitamin A deficiency in cattle. It did not correlate responses in plasma vitamin A with liver concentrations of vitamin A as does the current study. Furthermore, the data obtained with feedlot cattle are not applicable to young calves because feedlot cattle have a functional rumen whereas young calves essentially are monogastrics.

Results of the RDR assay with young calves are similar to results observed with humans. In humans (1, 2, 3), the RDR assay (with RDR values of ≥20% as an indication of deficiency) correctly identified individual patients with less than 20 μg of vitamin A/g of liver. In the current study, the RDR assay was an accurate indicator of the mean vitamin A status for groups of calves; however, values obtained with individual calves did not always indicate vitamin A status accurately. For 8 of 12 calves not supplemented with addi-

tional vitamin A, the RDR values were greater than 20% at either the 6- or 8-h postdosing sample. In contrast, only 2 of 41 calves supplemented with additional vitamin A had RDR values greater than 20% at 6-h postdosing, and both of those calves had RDR values less than 10% at 8-h postdosing. Because of possible problems in obtaining or analyzing plasma samples for vitamin A content from individual calves, the RDR assay may be most useful for diagnosing the vitamin A status of groups of calves at a similar age.

The mean concentration of retinol in plasma of calves at birth was similar to values reported by others (5, 6, 10, 21, 24). Calves typically are born with concentrations of retinol that are less than 10 μg/dl of plasma but without clinical signs of vitamin A deficiency. Concentrations of retinol in plasma of calves at birth less than 10 μg/dl do not necessarily indicate calves are deficient in vitamin A.

Based only on retinol concentrations in plasma, and following the practice of using guidelines established with older cattle (28), calves in all treatment groups at 4 wk of age had plasma concentrations of vitamin A that would be considered deficient (< 20 μg/dl). Mean concentrations of retinol in plasma were less than 15 μg/dl even for calves supplemented with 68,000 IU of vitamin A daily. The concentration of vitamin A in liver samples and the RDR assay, however, indicated that only calves not supplemented with additional vitamin A had low vitamin A status. Concentrations of retinol in plasma alone, therefore, were not an accurate indication of vitamin A status at 4 wk of age. These data indicate that there is a physiological mechanism in young calves that prevents plasma concentrations of retinol from exceeding 20 μg/dl. Feeding large amounts of vitamin A leads to increased storage in liver without elevation of plasma concentrations to levels considered normal for older cattle.

Other studies (10, 13, 24) determined concentrations of retinol in plasma of calves supplemented with vitamin A ranging from 10,000 (13, 24) to 30,000 (10) IU/d. All three studies reported retinol concentrations of less than 20 μg/dl of plasma when calves were 3 (24), 4 (13), or 6 (10) wk of age. Additionally, feeding colostrum and milk without additional vitamin A supplementation to calves increased plasma concentrations of retinol, but concentrations were less than 20 μg/dl at 6 wk of age (21). Plasma concentrations of retinol less than 20 μg/dl are normal for calves 6 wk of age or less and do not necessarily indicate deficiency. Supplementation with vitamin A to calves based only on plasma concentrations of retinol less than 20 μg/dl should be avoided. The RDR assay provides a more accurate indication of the vitamin A status of groups of calves than a single plasma sample and is less invasive than liver biopsies. Further studies are needed with calves of various ages to fully determine the usefullness of the RDR assay as a method for determining the vitamin A status of cattle.

CONCLUSION

This study is the first report of the use of the RDR assay to determine the vitamin A status of calves at 4 wk of age. It is also the first time results of the RDR assay were correlated with liver concentrations of vitamin A. As the amount of vitamin A supplementation increased, liver concentrations of vitamin A and plasma concentrations of retinol increased. Liver concentrations of vitamin A indicated that calves fed supplemental vitamin A had adequate vitamin A status. The RDR assay at 4 wk of age also indicated that calves fed supplemental vitamin A had adequate vitamin A status. Plasma concentrations of vitamin A, however, indicated that all calves were deficient at 4 wk of age based on current guidelines. Thus, guidelines recommending less than 20 μg of retinol/dl of plasma as an indication of vitamin A deficiency are not applicable for young calves. At 4 wk of age, liver biopsies and the RDR assay more accurately indicated the vitamin A status of calves compared with plasma samples. The RDR assay may be useful as a diagnostic tool to determine the vitamin A status of groups of calves. Further research is needed, however, with calves of varying ages.

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